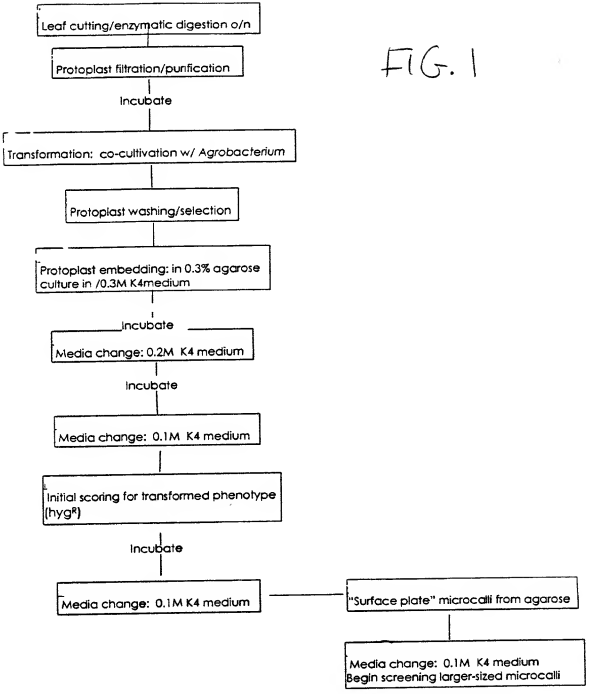


FIG. 1

Protoplast isolation and transformation flowchart.

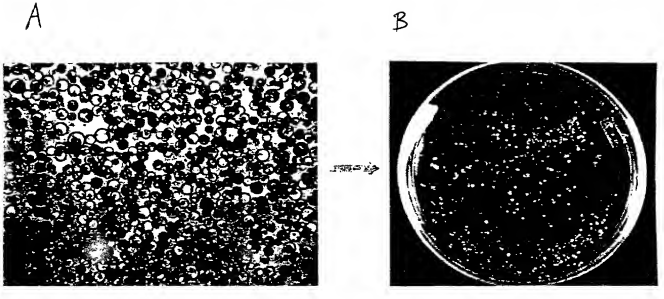


Figure 2. Freshly isolated protoplasts are mutagenized by ATM, subcultured and propagated to the stage of microcalli.

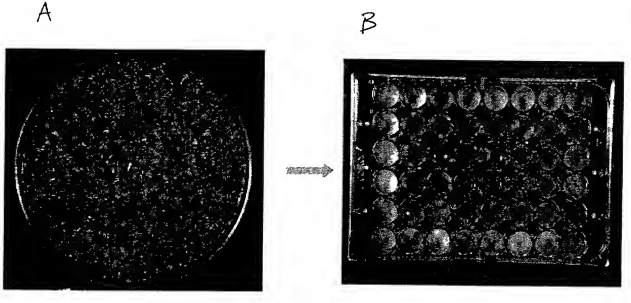


Figure 3, Individuals are sampled and a portion used to prepare extracts for assays. Steps are combined into a single procedure that establishes a library of viable, mutagenized calli.

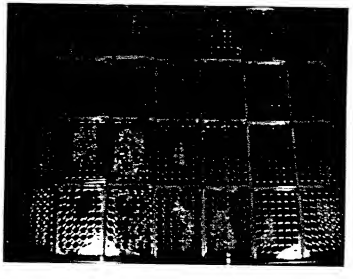


Figure 4. Part of an ATM "library" of callus cultures of *N. tabacum*. 1,344 individual clones with a random T-DNA insert - this represents one shelf of an incubator

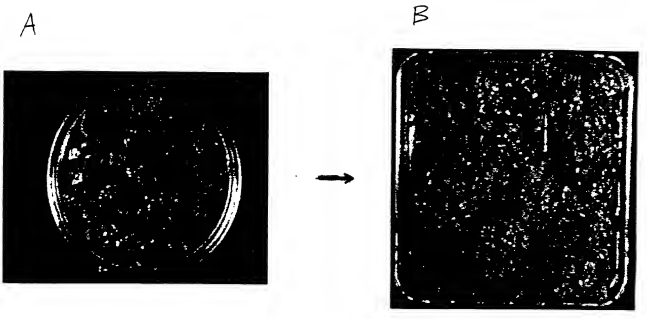


Figure 5, Tagged clonal daughter calli selected from a screen-positive "parent". Positive calli from secondary screen are regenerated to whole plants.

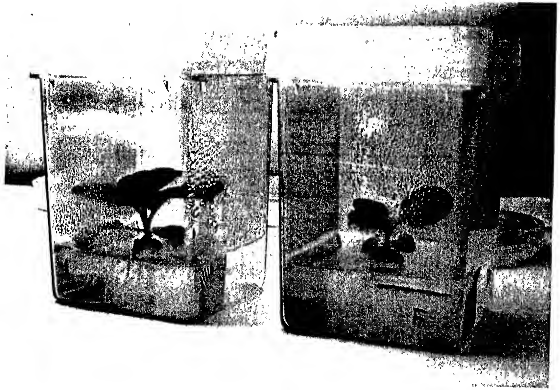


Figure 6. Magenta boxes containing intact plants regenerated from positive calli

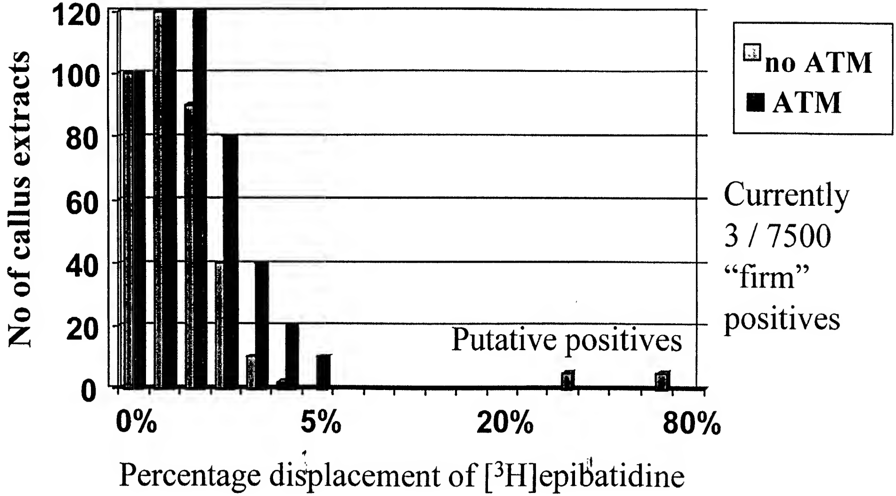


Figure 7. Screening of different populations of ATM'ed microcalli - illustrative data

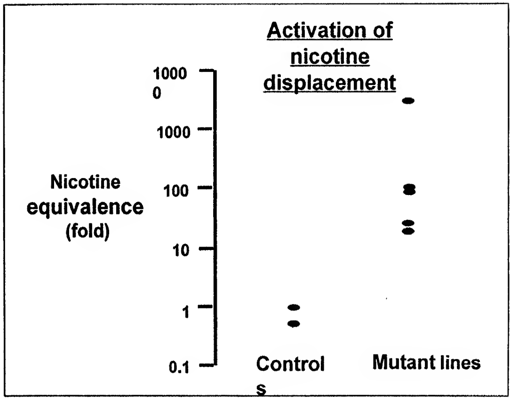


Figure 8. Culture extracts regarded as “positives” in relation to wild-type or transformed cultures. Clonal cultures are only regarded as “positives” when they continue to overproduce through several daughter generations.

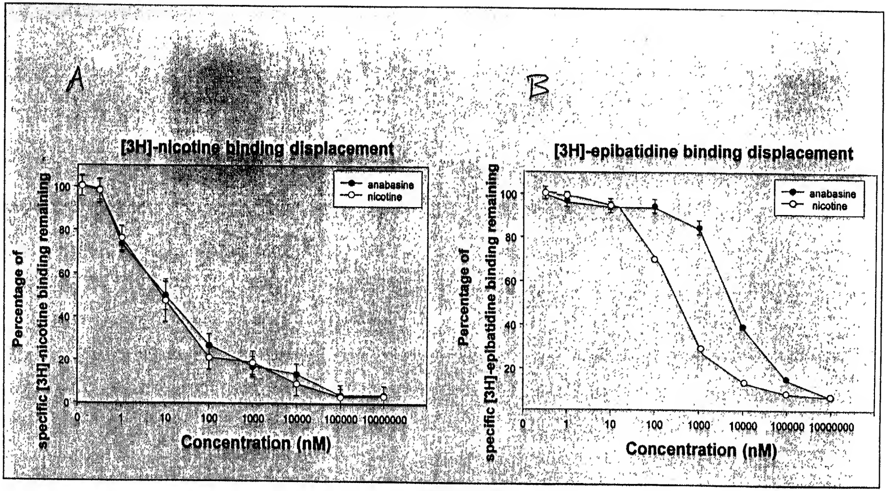


Figure 9A-9B. Initial activity characterization by displacement analysis.

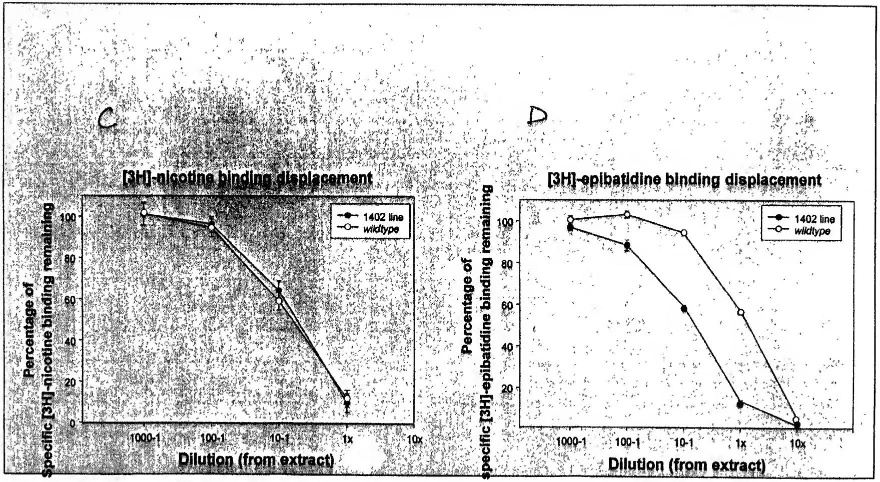
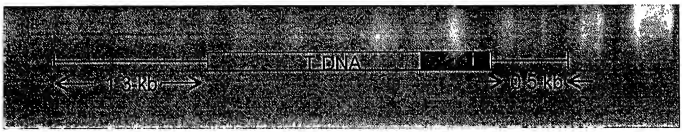


Figure 9C-9D. 1402 callus tissue extracts show distinct displacement profile from wildtype

- Genomic DNA adjacent to the activation-tagging element has been cloned from using plasmid rescue.



- 1.8-kb of plant genomic DNA has been recovered. Initial sequence analysis indicates homology with unknown expressed sequenced tags in soybean and barley roots.

- More extensive analysis is now underway using the cloned fragments as starting material (e.g., library probing, Northern analysis, etc)

Figure 10 Molecular characterization of line 1402